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Taxonomic study of thermophilic fungi in Thailand

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Key Words——thermophilic fungi; Thailand.

Summary

Thermophilic fungi were isolated from various substrates in Thailand. The thermophilic fungi studied represented 10 genera, 10 species and 3 unidentified species. The taxonomy of these fungi is discussed.

Introduction

Thermophilic fungi grow at temperatures between 20° and 50°C (Cooney and Emerson, 1964). This group of fungi functions in such tropical or subtropical industrial processes as cacao, oil palm, tobacco, guayule retting, enzyme and antibiotic productions and compost degradation (Cooney and Emerson, 1964). They utilize other agricultural waste products and contaminate various kinds of seeds and grains in storage.

These fungi, especially in the tropics, are capable of growing on various types of substrates such as mushroom compost, manure, animal nutrients and dung, industrial and agricultural waste products, self-heating hay, organic debris, birds' nests etc. Even soil exposed to the sun serves well for the growth of thermophilic fungi.

Tubaki and Tsuruta (1976) reported nine thermophilic species found on maize grains collected in Thailand, but they made no description of these species. This is the first taxonomic report with description of newly recorded thermophilic fungi in this country. The study of these fungi in detail is very important because of their significance in many fields. They are common in Thailand, and some have industrial and agricultural uses.

We isolated these thermophilic fungi from various substrates and identified them by their morphological characteristics.

Materials and Methods

The various substrates listed in Table 1 were collected from different parts of Thailand. A modification of Warcup's soil-plate method (Warcup, 1950); 20 ml of yeast-starch agar, yeast starch agar+Na-carboxymethyl cellulose or yeast extract glucose agar+one drop of a 10% aqueous solution of lactic acid was used in the isolation procedure. Moist chambers were used for the isolation from large and hard plant tissues. All the samples were incubated at 45°C for 5 days. The hyphal tips that developed were transferred to yeast starch agar slants as pure cultures. The isolated fungi were cultured on Petri dishes containing yeast extract glucose agar that were incubated at 15°, 20° and 50°C for 7–14 days. Fungi that grew at temperatures from 20° to 50°C were selected and maintained as pure cultures for our studies.

For our morphological and taxonomical studies, we cultured the selected strains on yeast extract glucose agar and yeast-starch agar, at a incubation temperature of 45°C for 4 to 5 days, after which slide preparations were and slide-culture techniques were made and examined under a compound microscopes. The freezing microtome technique was used for hard and resistant structures such as the ascocarp, the fungal culture being cut into small pieces and placed on a freezing microtome. After freezing, the specimen was cut into slices 5–7 μm thick, which were prepared as slides and observed under compound microscopes.

For scanning electron microscopy (SEM), a modification of Ellis' method (1981) was used. Small pieces of fungal cultures were fixed with 4% glutaraldehyde solution then rinsed with 2 or 3 changes of distilled water and dehydrated in a graded series of acetone. These specimens were passed through amyl-acetate and dried with a Hitachi HCT-2 Critical Point Dryer, after which, they were placed on adhesive tape on specimen holders and coated with gold with a JEOL JSM-1100 Ion Coater. These preparations were examined with a JEOL JSM-35CF SEM.

Results and Discussion

The thermophilic fungi isolated from 55 substrates represented 10 genera, 10 species and 3 unidentified species (Table 1 and Figs. 1–18). They belong to the Ascomycotina and Deuteromycotina.

ASCOMYCOTINA

Chaetomium thermophile La Touche var. *dissitum* Cooney et Emerson Figs. 1 & 2

Cooney and Emerson, Thermophilic Fungi, p. 68 (1964); Awao and Otsuka, Trans. mycol. Soc. Japan **14**: 222 (1973).

Colonies on yeast-starch agar grow well, are of irregular-shape, reach a diameter of 9 cm

Table 1. Thermophilic fungi isolated from 55 substrates from different locations in Thailand

Fungus	Type of substrates	Growth* Temperatures (°C)		
		15	20	50
Chaetomium thermophile var. *dissitum*	forest plant debris	−	+	+
Pseudeurotium sp.	decomposing sawdust, compost	−	+	+
Talaromyces thermophilus	composting starters	−	+	+
Thermoascus crustaceus	forest plant debris, forest soil	−	+	+
Thielavia terrestris	forest plant debris, sugar cane bagasses, rice straw	−	+	+
Humicola grisea var. *thermoidea*	straw mushroom compost (horse dung & lotus seed husks)	−	+	+
Humicola insolens	straw mushroom compost (horse dung & lotus seed husks)	−	+	+
Malbranchea sulfurea	corn cobs, sugar cane bagasses	−	+	+
Myceliophthora thermophila	forest plant debris, sugar cane bagasses	−	+	+
Myceliophthora sp. No. 1	forest plant debris	−	+	+
Myceliophthora sp. No. 2	forest soil	−	+	+
Scytalidium thermophilum	straw mushroom compost (horse dung & lotus seed husks), cow dung	−	+	+
Thermomyces lanuginosus	dried cassava chips, compost	−	+	+

* Incubated on yeast extract glucose agar.
+: growth, −: no growth.

within 4 days at 45°C, are white when young (24–48 h), but turn to grey, greyish green, brown or black at maturity. Mycelium 1–3 μm wide. Perithecia globose, subglobose or oval, 140–165 × 60–70 μm, with tortuous and dichotomously branched hairs. Asci 8-spored, cylindrical, usually produced in an obvious basal tuft. Ascospores globose or subglobose, 4–6 μm diam.

Habitat: Forest plant debris, Chanburi Province, March, 1983, (KM 0002).

This fungus grew well on all the agar media tested. The sizes of our isolates fit the original description given by Coony and Emerson (1964), except for their reporting smaller ascospores (4–6 μm vs. 6.5–9.5 μm).

Pseudeurotium sp. Figs. 3 & 4

Colonies on yeast-starch agar grow very well as they do on oatmeal agar and potato dextrose agar, reaching a diameter of 9 cm within 2 days at 45°C; convex with a smooth surface and entire margin, white at first turning to yellow, dull orange-yellow and brown at maturity. Aerial mycelium 1.5–8 μm in diam, septate, abundantly branched. Cleistothecia dull orange, formed within 24 h on both aerial and submerged mycelia, globose or subglobose, 90–120 μm in diam on aerial mycelium, but fairly irregular, 120–170 μm in diam, on submerged mycelium; peridium composed of brownish black polygonal cells in the outer layer and pale-colored, relatively round cells in the inner part. Asci globose to ellipsoid, dull orange, with 6–8 ascospores. Ascospores globose to ellip-

soid, unicellular, olive brown at maturity, 5–9×4–6 μm, with no germ pore.

Habitat: Decomposing sawdust for mushroom compost, Bangkok, May, 1983, (KM0009).

This fungus grew well on all the media tested and had abundant mycelial growth at 40–50°C; the minimum and maximum temperatures limits for growth were 35° and 55°C.

Talaromyces thermophilus Stolk Fig. 5

Stolk, Ant. Leeuwenh. **31**: 268 (1965); Cooney and Emerson, Thermophilic Fungi, p. 32 (1964); Awao and Mitsugi, Trans. mycol. Soc. Japan **14**: 150 (1973); Minoura et al., Trans. mycol. Soc. Japan **14**: 358 (1973).

St. anam. *Penicillium dupontii* Griffon et Maublanc

Colonies on yeast-starch agar reach a diameter of 3.5 cm within 4 days at 45°C, thickened at the center with a rough surface and entire margin, delicately floccose, white at first changing to cinnamon-lavender with a tint of green; dark brown drops of exudate formed in the central areas; reverse usually cinnamon-red.

Hyphae 2–3 μm wide. Conidiophores developed on side branches, usually perpendicular to the main hypha, 5–25×2–3 μm, smooth-walled, septate. Penicilli small and biverticillate, forming metulae and phialides. Conidia borne in long chains, pale yellow at maturity, elliptical or ovoid, smooth-walled, 4×1.5–3 μm. No teleomorph was present on the agar media.

Habitat: Decomposing starters, Bangkok, May, 1983, (KM0011).

Optimum growth occurred at 45–50°C, whereas the minimum temperature was 30°C.

Thermoascus crustaceus (Apinis et Chesters) Stolk Figs. 6 & 7

Stolk, Ant. van Leeuwenh. **31**: 272 (1965); Cooney and Emerson, Thermophilic Fungi, p. 42 (1964); Apinis, Trans. Br. mycol. Soc. **50**: 574 (1967); Awao and Otsuka, Trans. mycol. Soc. Japan **15**: 8 (1974).

St. anam. *Paecilomyces crustaceus* Apinis et Chesters

Colonies on yeast-starch agar reach a diameter of 7.3 cm within 3 days at 45°C, ochraceous, at first submerged mycelium closely spreading over the agar surface, later abundantly produced coarse-walled, branched aerial hyphae. Conidiophores 1000×10–15 μm, septate, coarse irregularly branched. Phialides irregularly arranged, elongate, flask-shaped, 10–20×3.7 μm. Conidia borne in long chains, hyaline to pale ochre, 1.5–3 μm in diam (vs. 4.5–7×2–2.5 μm, Cooney and Emerson, 1964). Cleistothecia formed within 4–6 days, pale yellow or pale orange-buff when young turning dull reddish brown in older stage, irregular shape. Asci subglobose or oval, evanescent, 8-spored. Ascospores hyaline, unicellular, oval to elliptical, 6–7×5–6 μm.

Habitat: Forest plant debris, Chonburi Province, February, 1983, (KM0012). Optimum temperature for the growth of this fungus is between 40° and 45°C, the minimum and maximum temperatures being 35° and 50°C. Our strain is very similar except

that the conidial size is much smaller.

Thielavia terrestris (Apinis) Malloch et Cain Figs. 8 & 9.

Malloch and Cain, Can. J. Bot. **50**: 66 (1972); Minoura et al., Trans. mycol. Soc. Japan **14**: 363 (1973); Samson et al., Trans. Br. mycol. Soc. **69**: 417 (1977).

Syn. *Allescheria terrstris* Apinis

St. anam. *Acremonium alabamensis* G. Morgan-Jones

Colonies on yeast-starch agar grow well, 4.8 cm in diam within 4 days at 45°C, white mycelium and smooth surface. The colony surface turns pink. Mycelium hyaline, septate, smooth-walled, 3.5–5.0 μm wide. Conidiophores produce one or several phialides (conidiogenous cells), occasionally lacking. Conidia hyaline, oval, with round apex and truncate base, 2.4×1–2 μm, usually in long chains or slimy heads. Cleistothecia formed at temperature below 40°C, immersed, black, globose, 100–200 μm in diam. Asci of irregular shape, scattered in the cleistothecial cavity, with 6–8 ascospores. Ascospores brown, oval, smooth-walled, 4–6×3–4 μm.

Habitat: Forest plant debris, Chanburi Province, Eastern Thailand, February, 1983, (KM 0001).

DEUTEROMYCOTINA

Humicola grisea Traaen var. *thermoidea* Cooney et Emerson Figs. 10 & 11

Colney and Emerson, Thermophilic Fungi, p. 79 (1964); Awao and Otsuka, Trans. mycol. Soc. Japan **15**: 12 (1974).

Colonies on yeast-starch agar reach 9 cm within 4 days at 45°C, greyish white when young, becoming dark grey at maturity; reverse grey to black. Conidia thallic, borne singly, globose or oval with thick walls, 8–16 μm in diam, yellowish brown when young and dark brown at maturity.

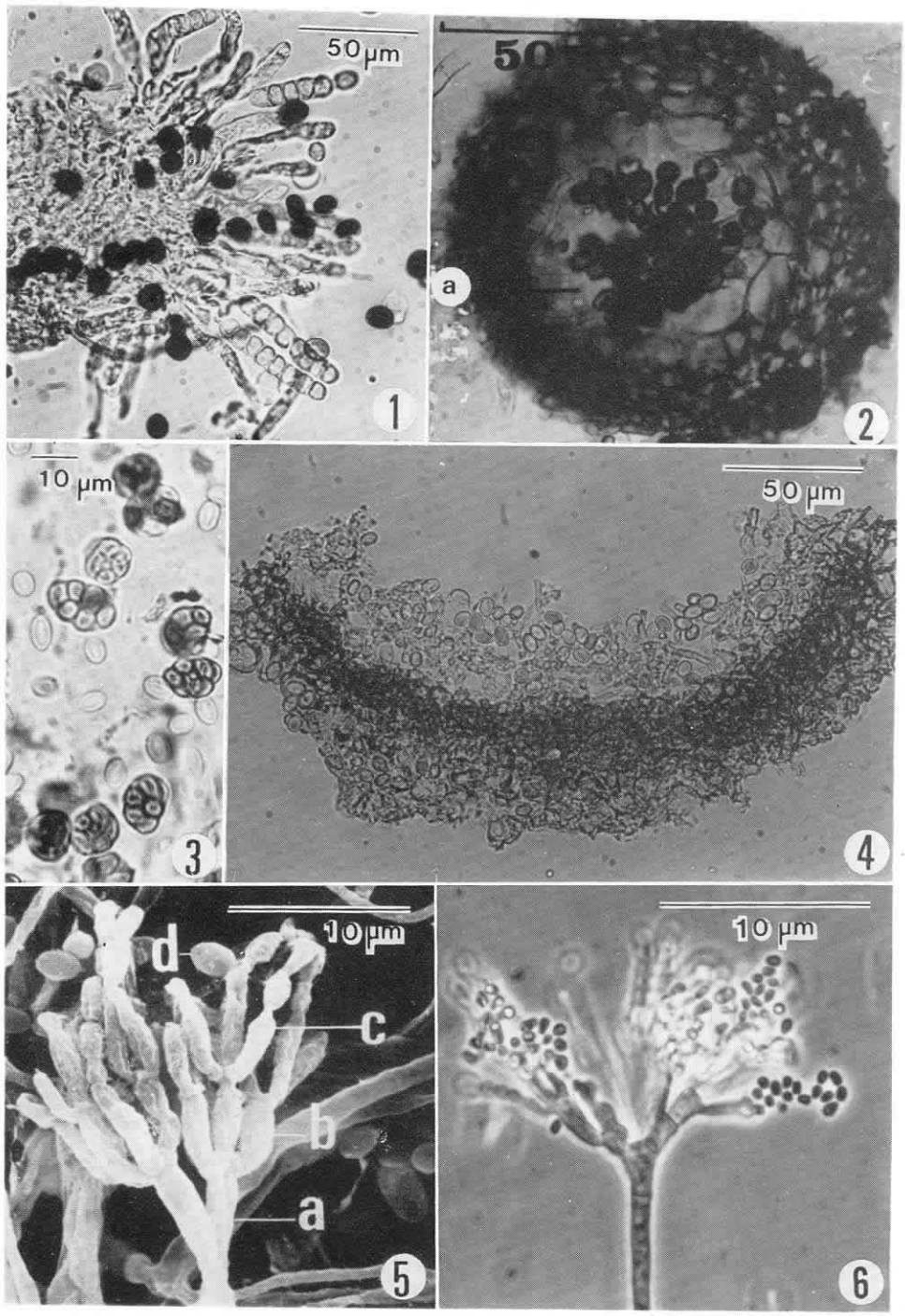
Habitat: Straw mushroom compost (horse dung and lotus seed husks), Bangkok, June, 1983, (KM 0003).

The size of the conidia in our strain is 9–15 μm in diam, whereas the original strain measured 12–16×8–12 μm (with the average of 8–16 μm in diam; Cooney and Emerson, 1964).

Humicola insolens Cooney et Emerson Figs. 12 & 13

Cooney and Emerson, Thermophilic Fungi, p. 78 (1964).

Colonies on yeast-starch agar, yeast extract glucose and oatmeal agar grow well, reaching a diameter of 9 cm within 4 days at 45°C, smooth surface and small fluffy mycelium, undulated and uneven margin, white or pale grey in 24–48 h; reverse dark colored due to the spore color. Conidia thallic, globose, 7–12 μm in diam, with a smooth, thick wall. Chlamydospores form in chains, intercalary.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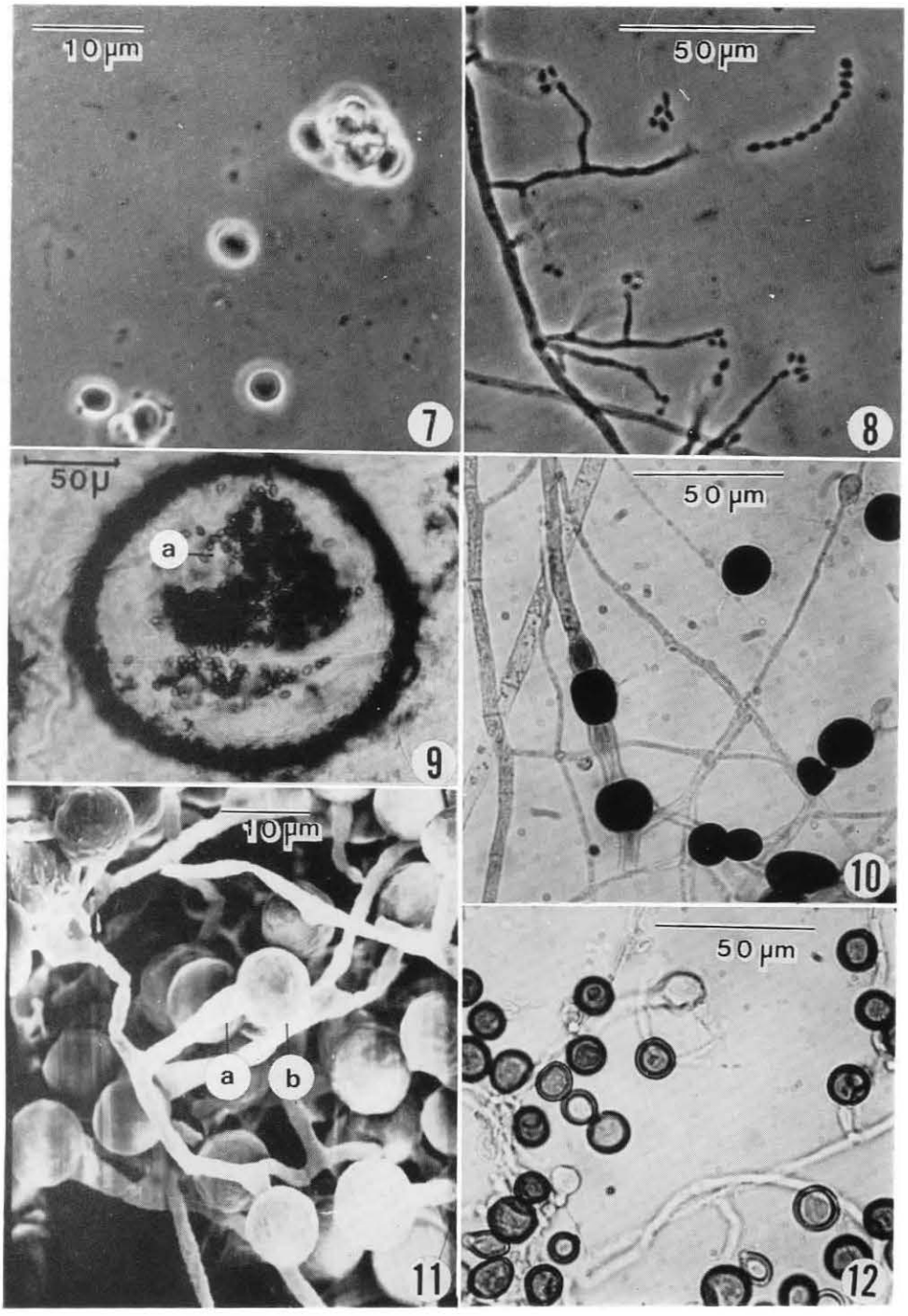
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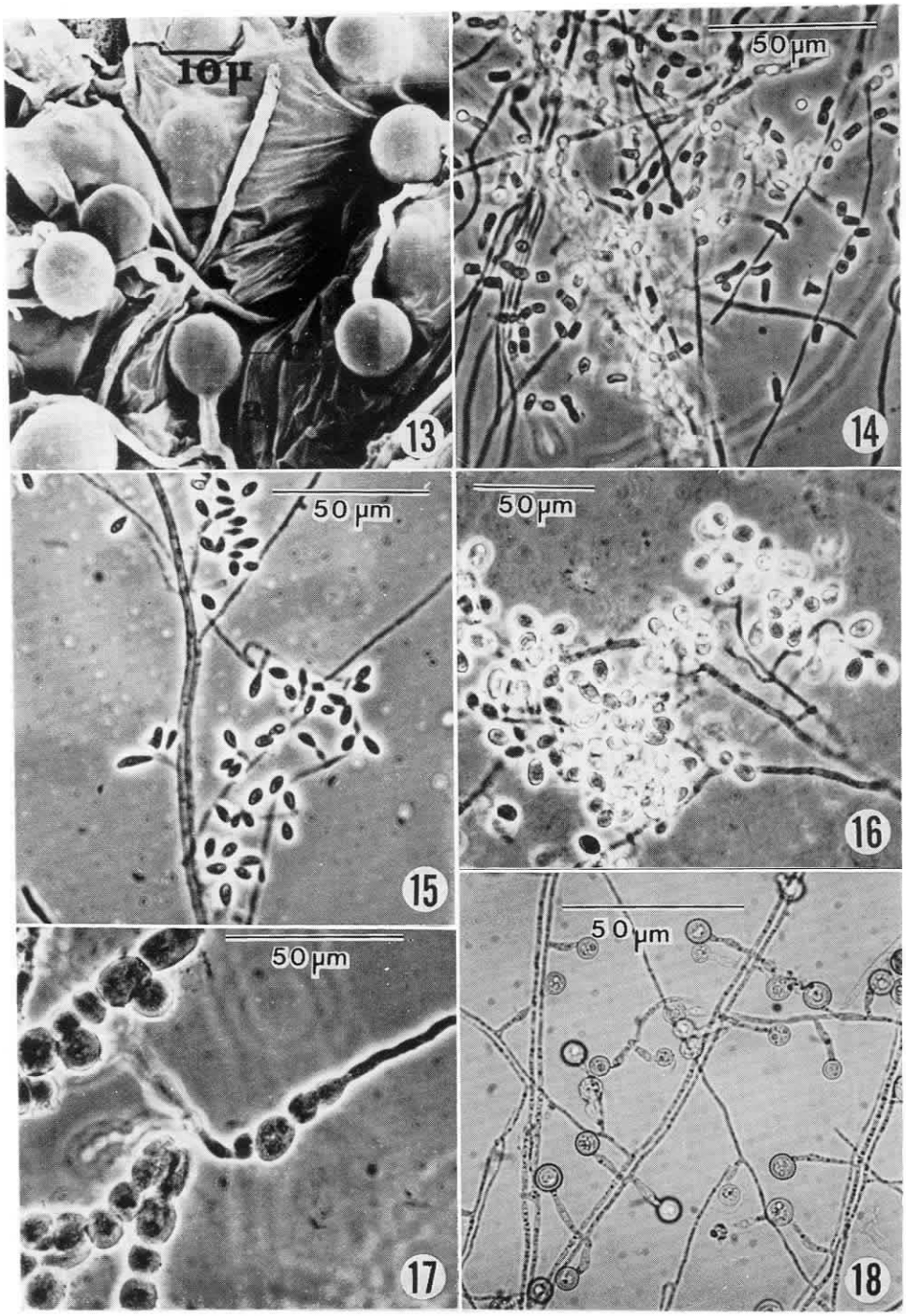
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Habitat: Straw mushroom compost (horse dung and lotus seed husk), Bangkok, March, 1983 (KM 0004).

Ellis (1982) studied the comparative morphology of *H. grisea* var. *thermoidea*, *H. insolens* and *Scytalidium thermophilum* (Cooney et Emerson) Austwick with SEM and TEM. These three fungi are very similar, therefore Ellis placed them in *Humicola-Scytalidium* complex. Awao and Otsuka (1974) treated this species as a probable synonym for *H. grisea* var. *thermoidea*.

Malbranchea sulfurea (Miehe) Sigler et Carmichael Fig. 14

Sigler and Carmichael, Mycotaxon **4**: 441 (1976); Cooney and Emerson, Thermophilic Fungi, p. 102 (1964); Awao and Otsuka, Trans. mycol. Soc. Japan **14**: 228 (1973).

Syn. *Malbranchea pulchella* Saccardo et Penzing var. *sulfurea* (Miehe) Cooney et Emerson

Colonies on yeast-starch agar grow fairly well as on oatmeal agar, yeast extract glucose agar and cornmeal agar, reaching a diameter of 5.7 cm in 4 days at 45°C, with a rough surface, white at first turning pale yellow, bright yellow then brown in the late stage; exudate brown, normally found on the colony surface; reverse dark brown to wine red. Mycelium white, 3–4 μm wide with a fertile hypha 10–100 μm long; its fertile tip usually curved into small loops or short helices which finally become block-like cells or arthroconidia. Arthroconidia 5–8 $\times$ 3–5 μm with rough, thick wall 0.6 μm thick, forms

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Figs. 1 & 2. *Chaetomium thermophile* var. *dissitum*:
1, asci and ascospores; 2, mature perithecium. (a, ascospores).
Figs. 3 & 4. *Pseudeurotium* sp.:
3, asci and ascospores; 4, a section of the peridial wall of cleistothecium.
Fig. 5. *Talaromyces thermophilus*:
Scanning electron micrograph of an anamorph (a, conidiophore; b, metula; c, phialide; d, conidium).
Figs. 6 & 7. *Thermoascus crustaceus*:
6, anamorph with branched conidiophore and conidia; 7, liberated ascospores.
Figs. 8 & 9. *Thielavia terrestris*:
8, anamorph; 9, a section of cleistothecium. (a, mature asci and ascospores).
Figs. 10 & 11. *Humicola grisea* var. *thermoidea*:
10, conidia; 11, conidia under SEM. (a, inflated conidiophore; b, conidium).
Figs. 12 & 13. *Humicola insolens*:
12, conidia and chlamydospores; 13, conidiophores and conidia under SEM.
Fig. 14. *Malbranchea sulfurea*:
liberated conidia.
Fig. 15. *Myceliophthora thermophila*:
conidia and conidiophores.
Fig. 16. *Myceliophthora* sp. No. 1:
conidia and conidiophores.
Fig. 17. *Scytalidium thermophilum*:
conidia in chains.
Fig. 18. *Thermomyces lanuginosus*:
conidia and conidiophores.

a powdery or dusty yellow mass at maturity after separation and release from the fertile hypha.

Habitat: Corn cob, Saraburi Province, Central Thailand, March, 1983, (KM0005).

Strain KM0005 of this fungus produces strong amylase activity for starch assimilation. In Malaysia, Kuthubutheem (1982) isolated this fungus from the atomosphere, oil palm seed and sugar cane bagass.

Myceliophthora thermophila (Apinis) van Oorschot Fig. 15

Van Oorschot, Persoonia **9**: 403 (1977); Van Oorschot, Stud. Mycol. **20**: 53 (1980).

Syn. *Sporotrichum thermophilum* Apinis

Chrysosporium thermophilum (Apinis) Klopotek

Teleom. *Thielavia heterothallica* Klopotek

Colonies on yeast-starch agar grow well as on other media, reaching a diameter of 9 cm within 3 days at 45°C; a rather rough surface, floccose, cottony or granular, or even powdery depending on the media used, white at first, the center turning cinnamon brown and darker with age; reverse pale yellow or green after 4 days of incubation. Conidia blastic, hyaline, oval, forming directly on a hypha or short conidiophore, usually in clusters on oval to clavate ampulliform swellings, 4–10×2–4 μm, with smooth or rarely warty wall.

Habitat: Forest plant debris, Chanburi Province, Feburuary, 1983, (KM0006).

Van Oorschot (1977) reported that Apinis described it as *Sporotrichum thermophilum* in 1963, but the generic name has been changed to *Chrysosporium* because of the lack of clamp connection. Von Arx (1973) suggested that *Chrysosporium* produces arthoconidia and aleurioconidia, whereas *Myceliophthora* produces blastoconidia. Van Oorschot (1977, 1980) reported that this fungus grew well at 30–36°C, in a temperature range of 24–54°C. The spore size of our isolate is close to that reported by Van Oorschot (4–10×2–4 μm vs. 4.5–11×3–4.5 μm), but our isolate had cellulase-producing ability, whereas van Oorschots isolate utilized keratin. No teleomorph was found in our study.

Myceliophthora sp. No. 1 Fig. 16

Colonies on yeast-starch agar grow rapidly, reaching a diameter of 9 cm in 3 days at 45°C; a rough surface and entire margin, fluffy and brownish from the periphery to the center. Conidia hyaline, 4×5 μm, with smooth or rough wall, borne on ampulliform swellings, singly or in clusters.

Habitat: Forest plant debris, Chanburi Province, February, 1983, (KM0007).

The present isolate differs from *M. thermophila* in having smaller conidia.

Myceliophthora sp. No. 2

Colonies on yeast-starch agar grow well, reaching a diameter of 9 cm in 4 days at 45°C, with smooth surface and entire margin, persistently white. Mycelium 1–5 μm

wide. Conidia hyaline, 4–6×3–4 μm, borne on ampulliform swellings, singly or in clusters, with smooth wall.

Habitat: Forest soil, Chanburi Province, February, 1983, (KM0008).

This fungus is very similar to *Myceliophthora* sp. No. 1 except for the colony color. It differs from *M. thermophila* in having smaller conidia.

Scytalidium thermophilum (Cooney et Emerson) Austwick Fig. 17

Austwick, N. Z. J. Agr. Res. **19**: 25 (1976); Cooney and Emerson, Thermophilic Fungi, p. 92 (1964); Awao and Otsuka, Trans. mycol. Soc. Japan **14**: 229 (1973)

Syn. *Torula thermophila* Cooney et Emerson

Colonies on yeast-starch agar reach a diameter of 9 cm within 3–4 days at 45°C, irregular, velvety with smooth surface, white at first gradually becoming black from the center to the margin because of conidial formation. Hyphae hyaline, septate, 2–5 μm wide. Conidia borne in chains as intercalary or terminal chlamydospores, dark brown, smooth-walled, usually spherical, 6–9 μm in diam, occasionally oval, 7–9×6–8 μm.

Habitat: Straw mushroom compost (horse dung and lotus seed husk), Bangkok, June, 1983, (KM0010).

Thermomyces lanuginosus Tsiklinskaya Fig .18

Tsiklinskaya, Ann. Inst. Pasteur Paris **13**: 500 (1899); Pugh et al., Trans. Br. mycol. Soc. **47**: 119 (1964); Kurata et al., Trans. mycol. Soc. Japan **7**: 99 (1966); Awao and Mitsugi, Trans. mycol. Soc. Japan **14**: 152 (1973); Ohtomo et al., Trans. mycol. Soc. Japan **16**: 289 (1975).

Syn. *Sepedonium lanuginosus* Griffon et Maublanc; *Humicola lanuginosa* (Griffon et Maublanc) Bunce

Colonies on yeast extract agar grow well as on potato-dextrose agar and oatmeal agar, reaching a diameter of 5 cm in 4 days at 45°C, white and felty at first, soon turning greenish grey from the center to the periphery, then dull dark brown at maturity; reverse wine colored.

Hyphae hyaline, septate, 2–3 μm in diam, producing masses of conidia on short, unbranched conidiophores (10–20 μm long) which arise at right angles on the hypha. Conidia borne singly at the tip of the conidiophore, 6–10 μm in diam, colorless when young turning dark brown at maturity with thick-wall. Both immature and mature conidia are very easily separated from the conidiophore, the detached conidia usually having a remnant of the conidiophore.

Habitat: Dried cassava chip, Chonburi Province, March, 1983, (KM0014)

Optimum temperature for growth 45–55°C, the minimum temperature being 30°C.

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摘　　要

タイ国産好高温菌類の分類学的研究

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タイ国は熱帯環境下にあるためカカオ，タバコ，ヤシ油，ゴムなどの諸生産過程で好高温菌の存在は物質分解に重要な意味をもつが，現在までまとまった研究はなかった．このため著者らはタイ国の落葉，キノコ栽培オガクズ，トウモロコシ，発酵食品，土壌など55の基質から好高温菌の分離・同定を試み *Chaetomium*, *Pseudeurotium*, *Talaromyces*, *Thermoascus*, *Thielavia* 諸属の子のう菌類，*Humicola*, *Malbranchea*, *Myceliophthora*, *Scytalidium*, *Thermomyces* 諸属の不完全菌類，計10属13種を記載した．